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Energy levels in resting and mitogen-stimulated human lymphocytes during treatment with FK506 or cyclosporin A in vitro

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Abstract

By employing microcalorimetry to assess overall metabolic activity in combination with other assays for specific metabolic events, we have investigated the influence of cyclosporin A and FK506 on the metabolic status of resting and mitogen-stimulated human peripheral lymphocytes. Both cyclosporin A and FK506 significantly reduced heat output from resting lymphocytes. This reduction could not be correlated with effects on DNA synthesis, lactate production, ATP levels or mitochondrial uptake of Rhodamine 123. These two drugs also potently reduced the increase in heat output seen during mitogen stimulation of lymphocytes. Both cyclosporin A and FK506 also prevented the increase in DNA synthesis, lactate production and ATP levels seen in response to mitogen stimulation. The increase in mitochondrial uptake of Rhodamine 123 during blastoid transformation was significantly reduced only by cyclosporin A. We ascribe the major part of the effects of these compounds to inhibition of the glycolytic pathway in both resting and mitogen-stimulated lymphocytes. These results indicate that the immunosuppressants cyclosporin A and FK506 exert other effects on lymphocytes than their well-established inhibitory action on calcineurin.© 1997 Elsevier Science B.V. All rights reserved.

Keywords: Microcalorimetry; Lymphocyte; Glycolysis; FK506; Cyclosporin A

1. Introduction

The immunosuppressive compound cyclosporin A (CsA) has been in clinical use for several years now. CsA has been shown to be of benefit in the treatment of allograft rejection, as well as for the treatment of certain disorders of autoimmune origin [1]. A few years ago FK506 was isolated from *Streptomyces tsukubaensis*. Although structurally unrelated, FK506 and CsA both cause potent suppression of T-cell activation by the same mechanism [2]. When bound to their respective immunophilins, these two compounds suppress transcription of the gene for IL-2 by inhibiting the enzymatic activity of calcineurin towards NF-AT [3].

Intensive investigation on the mode of action of CsA on lymphocytes and on its detrimental effects on other cell-types, e.g., renal cells [4], have generated a great deal of information concerning the different cellular processes affected by this compound. CsA has been reported to induce mitochondrial dysfunction in rat kidney cells [5]. Furthermore, CsA has been found to interfere with adenosine metabolism in

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murine B- and T-cells, thus leading to depletion of ATP [6]. Similar effects on peripheral rat lymphocytes during CsA administration in vivo have also been observed [7].

In addition, CsA has been found to block the release of mitochondrial matrix proteins through a Ca^{2+}-requiring pore, indicating beneficial effects of CsA in connection with reperfusion injury [8]. Other interesting effects of this drug include: depolarization of human peripheral lymphocytes [9], interference with plasma membrane phospholipid metabolism [10] and induction of cytoplasmic lipid droplets [11]. All these findings indicate that CsA has a larger number of biological targets than was believed to be the case when this drug was originally introduced into clinical practice.

FK506 has not yet been the subject of such thorough investigation. However, it was recently reported that FK506, like CsA, also induces respiratory dysfunction of mitochondria from rat kidney cells, but does not block the CsA-sensitive, Ca^{2+}-dependent pore [12]. FK506 has also been reported to induce mitochondrial dysfunction in rat enterocytes, thereby increasing intestinal permeability [13].

In the present study we have focused our attention on the effects of FK506 and CsA on resting and mitogen-stimulated human lymphocytes in vitro, with special emphasis on the bioenergetic changes these immunosuppressive compounds may give rise to.

2. Materials and methods

2.1. Chemicals

FK506 and Cyclosporin A were generous gifts from Fujisawa Pharmaceuticals (Osaka, Japan) and Sandoz Pharma (Basel, Switzerland), respectively. These compounds were dissolved in ethanol (1 mmol/l) in order to obtain stock solutions, from which aliquots were further diluted in culture medium. Concanavalin A (con A) was purchased from Pharmacia (Uppsala, Sweden), propidium iodide (PI) and Rhodamine 123 (R123) from Sigma Chemical (St. Louis, MO, USA) and [^{3}H]thymidine from Amersham (Buckinghamshire, UK). All other chemicals were of analytical grade and obtained from common commercial sources.

2.2. Cells

Human peripheral blood mononuclear cells were isolated from the buffy coats of healthy blood donors (The Blood Bank, Södersjukhuset, Stockholm, Sweden) by density centrifugation using Lymphoprep (Nycomed A/S, Oslo, Norway). The monocytes were removed from this preparation by allowing them to adhere to plastic dishes for 60 min. at 37°C. The non-adhering population was $>95\%$ lymphocytes, which were incubated under the different experimental conditions in RPMI-1640 culture medium supplemented with 10% heat-inactivated FCS and 50 μg/ml gentamicin. Cell counting was performed using a Coulter Cell Counter (Hialeah, FL, USA).

2.3. Bioactivity monitoring

A LKB 2277 Thermal Activity Monitor (TAM) (Thermometric AB, Järfälla, Sweden) of the thermopile heat conduction type was used for measurement of overall cellular heat production [14]. The instrument was equipped with three channels and was kept at 37°C. The signal was recorded on two twin-channel LKB 2210 potentiometric recorders (LKB, Bromma, Sweden).

The effects of the immunosuppresive drugs CsA and FK506 on the heat output from resting human lymphocytes were studied as follows: unstimulated cells (10^6/ml) were cultured in medium only or in medium containing 0.1 μM CsA or FK506 in 75 cm^2 plastic bottles for 5 days. Two ml cell suspension from each flask were transferred to the reaction ampoule and 2 ml medium to the reference ampoule, leaving a 3 ml gas-phase. Heat output was then recorded continuously for the following 24 h.

The steady-state value was normally obtained within 1 h and was stable throughout the remainder of the 24-h incubation period. After 24 h, the cells were removed from the ampoules and counted. New cells from the flasks were then transferred to the reaction ampoules and these incubations were repeated for five consecutive 24-h intervals.

The inhibitory effect of these drugs on heat output from proliferating cells was also tested using the calorimeter, as described previously [15]. In these experiments 10^6 cells in 4 ml RPMI were incubated with 0.1 μM immunosuppressant together with the

mitogen con A (10 μg/ml). The cell suspensions were then immediately transferred to the ampoules, leaving a 1 ml gas-phase, and inserted into the calorimeter. The heat output was continuously monitored for the following 60 h.

In the case of con A-stimulated lymphocytes the values are presented as total heat output from the 10^6 cells originally incubated in 4 ml medium. The number of cells in the ampoule after the 60-h incubation period was still 10^6.

Judging from published values for oxygen [16] and glucose [17] consumption by resting, as well as, by proliferating lymphocytes, the levels of glucose and oxygen present during the incubations conducted here were far from limiting.

2.4. DNA synthesis

Cell growth was monitored as [^{3}H]thymidine incorporation, as previously described [18]. One hundred μl medium containing 10^5 cells were cultured in 96-well microtiter plates (Falcon, Becton Dickinson, Lincoln Park, NJ, USA) in 5% CO_2 at 37°C. To each well, 1 μCi [^{3}H]thymidine was added during the last 4 h of incubation. [^{3}H]Thymidine incorporation in both resting and con A-stimulated cells was measured for three reasons: to detect any unexpected activation of the resting cells; to confirm activation of the cells treated with con A; and to assess and the effects of CsA and FK506 on DNA synthesis.

2.5. Lactate production

Measurements of the lactate content in the media from resting and mitogen-stimulated cells, cultured in a 5% CO_2-incubator at 37°C, were performed using a colorimetric procedure performed according to the manufacturer's instructions (Sigma Chemical, St. Louis, MO, USA). Absorption at 540 nm was measured using an Hitachi U3210 spectrophotometer (Tokyo, Japan). Resting or con A-stimulated cells were cultured in plastic bottles (10^6/ml) for 96 h. Once every 24 h, 1 ml of medium was collected, cleared by centrifugation and stored at −80°C for later analysis of lactate levels.

2.6. ATP assay

The intracellular content of ATP in resting and con A-stimulated cells was determined using a luciferase-based luminometric procedure performed according to the manufacturer's instructions (Bio-Orbit, Turku, Finland). Analyses were performed on an LKB 1250 luminometer and the output recorded on an LKB 2210 potentiometric recorder (LKB, Bromma, Sweden). A standard curve was generated for each set of experiments using a standard ATP solution (Bio-Orbit, Turku, Finland).

2.7. Flow cytometry

A FACSCalibur (Becton-Dickinson, San Jose, CA, USA) equipped with a 488 nm air-cooled argon-ion laser was used for all flow cytometric analyses. Data from 5000 events were collected for each sample. Off-line analysis was performed using the CellQuest software (Becton-Dickinson, San Jose, CA, USA).

2.8. Mitochondrial membrane potential

To evaluate the effects of drug treatment on mitochondrial membrane potential, 10^6 cells in a 1 ml suspension were stained with 1 μg Rhodamine 123 (R123) [19–21]. Uptake of this dye after a 10-min. incubation at 37°C was measured by flow cytometry. Resting and con A-stimulated cells were cultured in plastic bottles (10^6/ml) for 96 h. Once every 24 h, 1 ml aliquots of cell suspension were removed and stained with R123.

2.9. Cell viability

Viability was also monitored by flow cytometry using propidium iodide (PI) to detect the increased plasma membrane permeability of dead cells, as described by Sasaki et al. [22]. Cells were stained with 0.5 μg PI/ml and analyzed within the subsequent 30 min.

2.10. Expression of antigens associated with activation

HLA-DR was quantified at certain stages during the incubations in order to confirm that the resting lymphocytes had not been activated. A FITC-conjugated mouse anti-human HLA-DR monoclonal antibody (Dakopatts A/S, Glostrup, Denmark) was employed for this purpose.

2.11. Statistics

Statistical analysis was performed using the alternate Welch *t*-test.

3. Results

3.1. Calorimetric monitoring of resting and con A-stimulated cells

Resting lymphocytes demonstrated a heat output of 1.46 ± 0.13 pW/cell after 24 h of incubation (Fig. 1A). This value increased during the first days of incubation, reaching 2.2 ± 0.3 pW/cell after 72 h and then declining to 1.73 ± 0.23 after 144 h. FK506- and CsA-treated cells showed a markedly depressed heat output in comparison to control cells. After 24 h of incubation, the heat output from CsA- and FK506-treated cells were 1.3 ± 0.11 and 1.45 ± 0.22 pW/cell, respectively. These values remained fairly constant at a level ranging between 1.13 and 1.54 pW/cell. Thus, the heat output from FK506-treated cells differed significantly from that of untreated cells after 48, 72 and 96 h of incubation; whereas heat output from CsA-treated cells was significantly lower than the control value after 72 and 144 h of incubation.

Heat-output from con A-stimulated cells after 5 h of monitoring was 3.13 ± 0.37 μW, while the corresponding values in the prescence of CsA or FK506 were 3.45 ± 0.38 and 2.45 ± 0.15 μW, respectively (Fig. 1B). Heat output from the con A-stimulated cells in the absence of immunosuppressant then gradually increased and reached a maximal value of 11.73 ± 2.03 μW after 55 h of incubation, giving a net increase of 8.6 μW. Cells treated with CsA, however, demonstrated their maximal heat output of 4.65 ± 0.64 μW after 45 h, which gives a net increase of 1.2 μW (15% of the control increase). FK506-treated cells also reached their maximal heat output of 4.1 ± 1.05 μW after 45 h, a net increase of 1.65 μW (20% of the control increase).

3.2. DNA synthesis in resting or con A-stimulated cells

As can be seen from Fig. 2A, no DNA synthesis could be detected in unstimulated cells or in resting cells treated with FK506 or CsA. As expected, mitogen stimulation of the lymphocytes induced DNA synthesis, reaching a maximal rate of thymidine incorporation (92500 ± 5700 cpm) after 75 h of incubation. CsA- and FK506-treated cells incorporated only 35% and 33%, respectively, of the control value at this same time-point (Fig. 2B).

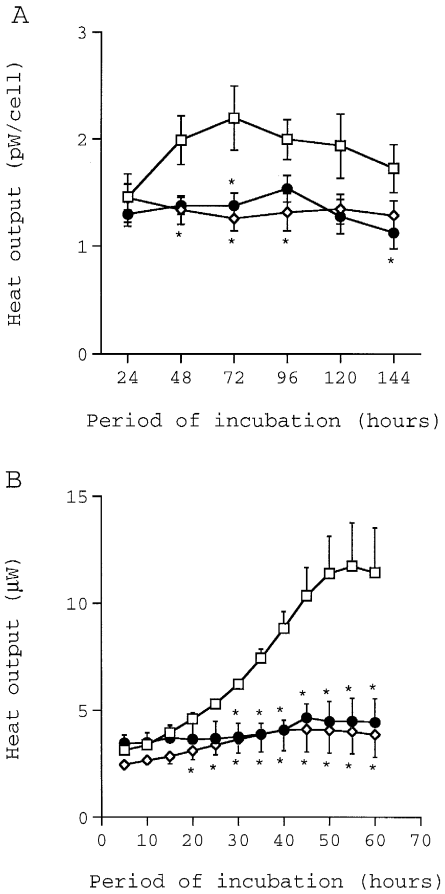

Fig. 1. Heat output of resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: heat output from resting lymphocytes ($2 \cdot 10^6$ cells in 2 ml of medium) (□) and lymphocytes incubated in the prescence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. B: heat output from con A (10 μg/ml) stimulated lymphocytes (10^6 cells in 4 ml of medium) (□) and stimulated cells in the prescence of 0.1 μmol/l CsA (●) or FK506, respectively (◇). The values are means ± S.E.M., $3 \leq n \leq 4$. * Value significantly different from the corresponding control value at the level of $P \leq 0.05$.

3.3. Lactate production by resting and con A-stimulated lymphocytes

Untreated cells produced barely detectable amounts of lactate during 96 h of incubation (Fig. 3A). When

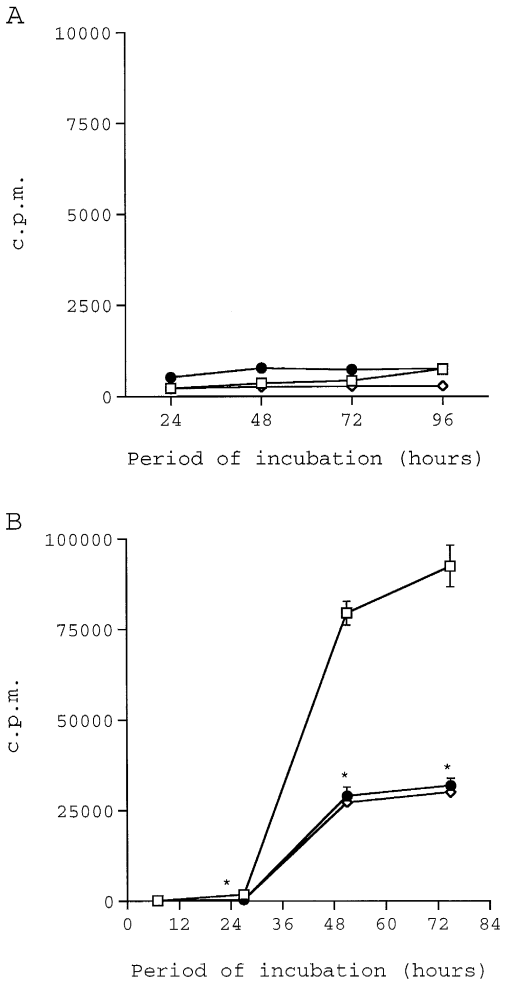

Fig. 2. Thymidine incorporation in resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: [^{3}H]thymidine incorporation into resting lymphocytes (□) and cells in the prescence of 0.1 μmol/l CsA (●) or 0.1 μmol/l FK506 (◇), respectively. B: [^{3}H]thymidine incorporation into con A (10 μg/ml) -stimulated lymphocytes (□) and stimulated cells in the presence of 0.1 μmol/l CsA (●) or 0.1 μmol/l FK506 (◇), respectively. The values are means ± S.E.M., $5 \leq n \leq 8$. * Values for both CsA- and FK506-treated cells significantly different from the control value at the level of $P \leq 0.0001$.

lymphocytes were stimulated by mitogen, increasing amounts of lactate accumulated continuously in the medium. In mitogen-stimulated cells, the levels of lactate increased 16-fold between 24 and 96 h of incubation. In the prescence of FK506, this increase was 8-fold. CsA was even more potent in this respect, reducing the increase in amount of accumulated lactate to only 6.7-fold (Fig. 3B).

3.4. ATP levels in resting and con A-stimulated cells

Analysis of the cellular ATP content of the resting cells after 24 h in the calorimeter revealed that untreated lymphocytes contained 540 ± 37 pmol/10^6 cells and that this level decreased slightly during the course of the experiment, being 480 ± 39 pmol/10^6 cells after 96 h of incubation. Similar results were obtained with FK506- and CsA-treated cells (Fig. 4A). No major increase in ATP content in response to con A-stimulation could be detected after 48 h. After

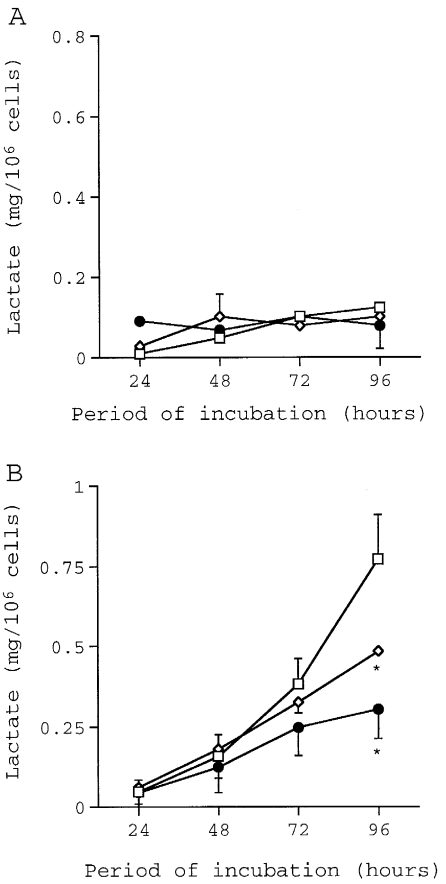

Fig. 3. Lactate release from resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: accumulation of lactate from resting lymphocytes (□) and resting cells incubated in the prescence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. B: accumulation of lactate from con A (10 μg/ml) stimulated lymphocytes (□) and stimulated cells in the prescence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. The values are means ± S.E.M., $n = 3$. * Value significantly different from that of the control cells at the level of $P \leq 0.03$.

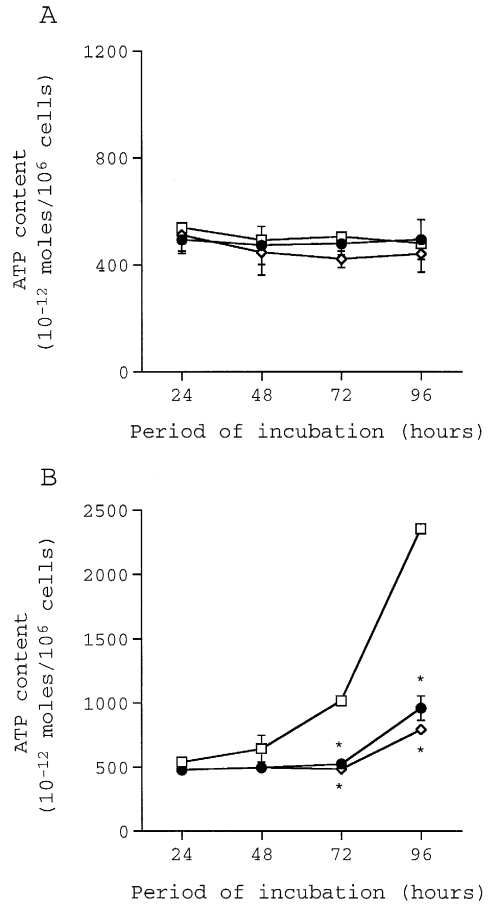

Fig. 4. ATP contents of resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: ATP contents of resting lymphocytes (□) and of resting cells incubated in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. B: ATP contents of con A stimulated lymphocytes (□) and of stimulated cells in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. The values are means ± S.E.M., $n = 4$. * Value significantly different from that of the control cells at the level of $P \leq 0.006$.

72 h of incubation, the ATP contents had increased 2-fold over the basal level (1020 ± 47 pmol/10^6 cells) and after 96 h this increase was 4-fold (2350 ± 36 pmol/10^6 cells) (Fig. 4B). Treatment with FK506 or CsA clearly reduced this increase in the level of intracellular ATP. ATP levels in treated and untreated con A-stimulated cells were similar after 24 h of incubation. After 72 h of con A-stimulation in the prescence of CsA or FK506, cellular ATP contents had still not increased over basal values and were significantly lower than for the mitogen-stimulated control cells. After 96 h of con A-stimulation in the prescence of CsA or FK506, however, the cellular ATP contents had increased 2-fold and 1.7-fold, respectively, thus still differing from the mitogen-stimulated control cells in a statistically significant manner.

3.5. Mitochondrial membrane potential

The distribution of R123 fluorescence in resting and con A-stimulated cells after 72 h in culture is shown in Fig. 5. As can be seen, resting cells demonstrated relatively homogenous fluorescence, with a single peak containing appproximately 95% of the events (M1, Fig. 5A). During mitogen stimulation, the uptake of R123 increased in some lymphocytes and, consequently, these cells gave rise to a second peak with a higher mean fluorescence intensity (M2, Fig. 5B). These latter cells are denoted $R123^{bright}$. The size of the $R123^{bright}$ population is shown in Fig. 6. The size of this population in untreated resting lymphocytes ranged between 4.1 ± 1.2 and 6.2 ± 0.17% of the total number of cells during the 96-h time period studied. Although the size of this population increased to 9.6 ± 4.5 and 10.4 ± 3.0% after 96 h in CsA- and FK506-treated cells, respectively (Fig. 6A), these increases were not statistically significant. During con A-stimulation the size of the $R123^{bright}$ population rapidly increased to 20.8 ± 2.8% after 24

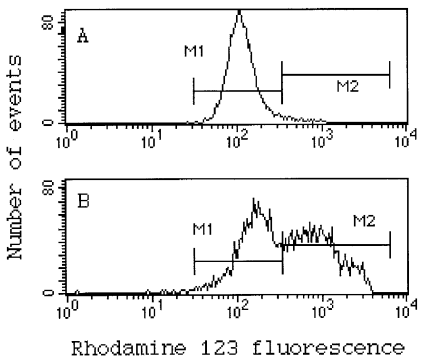

Fig. 5. R123 fluorescence from resting and con A-stimulated lymphocytes. A: typical distribution of R123 fluorescence from resting lymphocytes incubated for 72 h in vitro. The majority of the cells are recovered within the M1 region. B: typical distribution of R123 fluorescence from lymphocytes stimulated with con A (10 μg/ml) for 72 h. Approximately 50% of the cells exhibit an increase in R123 fluorescence and are found in the M2 region, referred to as the $R123^{bright}$ population.

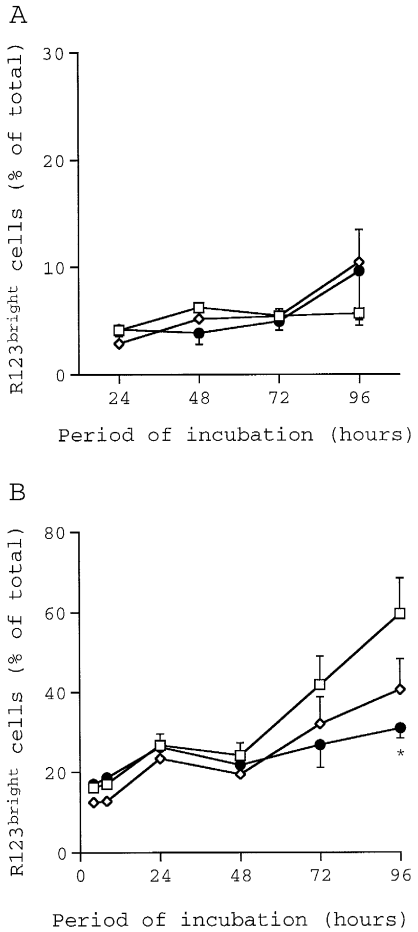

Fig. 6. R123 fluorescence from resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: size of the R123^{bright} population in resting lymphocytes (□) and in resting cells in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. B: size of the R123^{bright} population in lymphocytes stimulated with con A (10 μg/ml) (□) and in stimulated cells in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. The values are means ± S.E.M., $n = 3$. * Value significantly different from the control value at the level of $P = 0.03$.

h of incubation and remained at this level during the next 24 h. After 72 h of incubation, 41.9 ± 7.1% of the cells were found in this population and after 96 h 59.6 ± 8.9% of the cells showed increased uptake of R123. The corresponding values for CsA- and FK506-treated cells were similar to that of untreated con A-stimulated lymphocytes during the first 48 h of incubation. After 72 h, however, the sizes of the R123^{bright} populations were 26.8 ± 5.7 and 32.1 ± 6.8% for CsA- and FK506-treated cells, respectively. These values were not significantly different from the mitogen-stimulated control cells. After 96 h the corresponding values were 31.0 ± 2.5% and 40.6 ± 7.7%, respectively (Fig. 6B). Thus, CsA, but not FK506, significantly reduced the size of the R123^{bright} population compared to the mitogen-stimulated control cells after 96 h of incubation.

3.6. Cell viability

The viability of resting lymphocytes, as assesed by PI staining, was the same for FK506- or CsA-treated

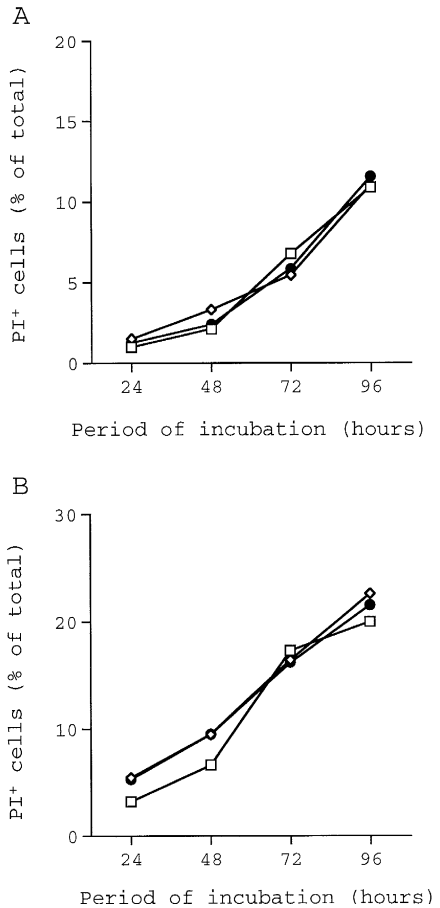

Fig. 7. Uptake of propidium iodide by resting and con A-stimulated lymphocytes in the absence and presence of CsA or FK506. A: uptake of propidium iodide by resting lymphocytes (□) and by resting cells in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. B: uptake of propidium iodide by lymphocytes stimulated with con A (10 μg/ml) (□) and by stimulated cells in the presence of 0.1 μmol/l CsA (●) or FK506 (◇), respectively. These data are from one representative experiment of three which gave very similar results.

cells and control cells. After 24 h of incubation only 1–1.5% of the cells were permeable to the dye and this value increased to 10.9–11.6% after 96 h (Fig. 7A). Con A stimulation of the cells resulted in a somewhat lower viability, i.e., 3.2% dead cells after 24 h, increasing to 20% after 96 h (Fig. 7B). Again, FK506 and CsA were without effect on the viability of mitogen-stimulated lymphocytes

3.7. Analysis of activation of resting cells

Analyses of HLA-DR expression and uptake of [^{3}H]thymidine by resting cells were performed in order to be certain that non-stimulated lymphocytes were not activated by some other stimulus during the culture period. Neither of these tests gave any indication of activation of the resting cells (Fig. 2A + data, not shown).

4. Discussion

Quiescent lymphocytes have very low metabolic activity, which is reflected in a low heat production compared to other cell types [23]. Upon mitogen stimulation, a number of metabolic processes are enhanced, leading to an increase in heat output [15,24,25]. The most prominent feature is an increase in lactate production [26], reflecting an increase in the contribution of glycolysis to overall ATP synthesis from 4% to 61% after 48 h of mitogen stimulation [16].

Oxidative ATP production, on the other hand, is not up-regulated during blastogenesis as long as glucose is available (the Crabtree effect) [16]. If, however, glucose is not available, oxygen consumption increases and the increase in ATP production is totally accounted for by oxidative pathways [16]. Even though the oxidative ATP production does not increase, the mitochondrial uptake of R123 increases during blastogenesis [27,28]. This may reflect an increase in mitochondrial mass per cell and/or an increase in mitochondrial membrane potential.

Unexpectedly, we found that heat output from the untreated resting lymphocytes was significantly elevated after 72 and 96 h compared to the value obtained after 24 h. A similar finding has also been reported by Gorski and Levin [24], who found an increase, although not significant, in heat output from resting lymphocytes cultured in vitro. We also found that the elevation of heat production was significantly prevented by both CsA and FK506.

It is difficult to explain this increase in resting heat production and, consequently, the effect(s) of the immunosuppresants, since it was not reflected in any of the other parameters tested. This is in agreement with the results of Wang and coworkers [29], who could not find any increase in glucose consumption, lactate production, RNA or DNA synthesis in resting lymphocytes during 120 h of culture in vitro. Marjanovic and coworkers [30] have, however, reported that glycolytic enzymes were induced in resting lymphocytes during 48 h of culture in medium in vitro, although no increase in lactate production could be detected. These investigators ascribed this induction to stimulation of the cells by endogenous serum factors present in the medium. Addition of con A to these cells gave, however, no additional induction of glycolytic enzymes.

Such enzyme induction in resting lymphocytes may lead to an increased rate of glycolysis, at least in a subpopulation of the cells, which is reflected in the rise in heat production. If this is the case, prevention of this increase in heat production by CsA and FK506 could reflect their ability to interfere with glycolysis. There are, of course, other exothermic processes that might be up-regulated in resting lymphocytes, e.g., mitochondrial respiration. We have not been able to find any reports of elevated respiration in resting lymphocytes during culture in vitro. Other reports indicate that both CsA and FK506 inhibit mitochondrial respiration in other cell types [12,13,31,32]. If such inhibition also occurs in lymphocytes, this may explain, at least in part, the decrease in basal heat output caused by FK506 and CsA, especially in the light of the fact that oxidative metabolism is the major source of ATP and heat in resting lymphocytes [16,25].

Here, we could not confirm the reported decrease in the cellular levels of ATP upon CsA treatment of non-stimulated lymphocytes [6,7]. This discrepancy may be explained by the very different conditions used by these other investigators.

Human lymphocytes responded with a profound increase in heat output (from 3 μW to 12 μW/10^6 cells) after 55 h of con A stimulation. In contrast, the

heat output from CsA- and FK506-treated con A-stimulated cells did not increase much above the initial value during the entire incubation period in the calorimeter. The increases in DNA synthesis and intracellular ATP content in con A-stimulated lymphocytes came after the increase in heat output. These findings confirm the results of Yamamura and coworkers [33], who used calorimetry to monitor heat output during the cell cycle of a synchronized mouse breast cancer cell line. These investigators found that heat output increased throughout the G1, S and G2 phases, followed by a rapid decrease as soon as the cells entered the M phase and the cell number started to increase.

Lactate production in con A-stimulated lymphocytes was also depressed by both CsA and FK506, with CsA being more potent in this respect. There are obviously different mechanisms controlling glycolysis in different cell types, since lactate production by the rat enterocytes studied by Madsen and coworkers was totally unaffected by FK506 [13]. The findings of Wollberg and Nelson [34], on the other hand, agree with ours, since these investigators found FK506 to inhibit enolase and LDH isozymes in mitogen-stimulated lymphocytes. CsA has also been reported to interfere with glucose metabolism in lymphocytes, antagonizing the effects of phenylephrine, but having no effect on lactate production itself in resting cells [35].

Our results on mitochondrial membrane potential confirm the findings of Koponen and coworkers [28], who observed a decrease in mitochondrial uptake of R123 during treatment of mitogen-stimulated lymphocytes with CsA. However, in the present study, FK506 did not significantly reduce the increase in R123 uptake associated with mitogen stimulation.

Our data indicate that CsA and FK506 both inhibit the induction of glycolysis caused by mitogen stimulation of lymphocytes as well as, possibly, in resting cells; and that it is this inhibitory effect that is reflected in the smaller increases in heat output, DNA synthesis, lactate production and ATP content. Of course, the possibility remains that the effects on resting cells may involve alterations in oxidative phosphorylation, especially in the light of the fact that lactate production in these cells is apparently not affected by exposure to CsA or FK506, although these measurements were close to the limit of detection for this assay. The depression of overall metabolism from the resting cells by these compounds, as measured by microcalorimetry, may reflect inhibition of different exothermic processes not possible to identify with specific assays. Induction of glycolytic enzymes and an increase in aerobic glycolysis have been found to be more or less a prerequisite for DNA synthesis [29]. We and others have also found that inhibitors of glycolysis impair the lymphocytic response to mitogen to a much larger extent than do inhibitors of mitochondrial respiration, further illustrating the importance of the glycolytic pathway during lymphocyte stimulation [26,36].

Since little information is available on the mechanisms underlying the induction of these metabolic events in mitogen-stimulated lymphocytes, we cannot say whether the inhibition of calcineurin by these two immunosuppressive drugs is involved in their effects on the metabolic parameters monitored here. These effects may also reflect a separate chain of events, involving an inhibitory action of these drugs on the as-yet-elusive natural cellular role(s) for the immunophilins [8,37–41].

In conclusion, there would seem to be many, as-yet-unidentified cellular events which are affected by CsA and FK506. Further research will provide information which may also allow us to explain the side-effects of these drugs, thereby providing a basis for selecting the treatment of choice in clinical practice.

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